Molecular detection and characterization of potyvirus isolates infecting commercially important Crop plants from Shekhawati region of Rajasthan.

A
SYNOPSIS
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INTERODUCTION:

The *Potyvirus* genus is the largest genus of the family *Potyviridae*. The Potyviridae (named after *Potato virus* Y) is the largest family of plant viruses currently recognized containing 218 definite and tentative species (Berger *et al.*, 2005). The family Potyviridae is classified into six genera, Potyvirus, Bymovirus, Rymovirus, Ipomovirus, Macluravirus and Tritimovirus. These genus are distinguished on the basis of their genome organization, vector transmission and genome sequence (Berger *et al.*, 2005). Virions of Potyvirus range in length from ~700 to 900 nm and encapsidate a monopartite, single stranded RNA genome ~10 kb, characterized by a 5' untranslated region (5' UTR), a single open reading frame (ORF) and a 3' UTR which has a polyadenylated (poly A) tail. The ORF encodes a single large Polyprotein that is subsequently processed into ten functional proteins (Adams *et al.*, 2005). Potyviruses are mainly transmitted by aphids in a non-persistent manner and infect a wide range of crops in which they causes significant losses. Although worldwide in their distribution, they are most prevalent in tropical and subtropical countries (Shukla *et al.*, 1998).

Members of this genus may cause significant losses in agricultural, pastoral, horticultural and ornamental crops Potyviruses can induce a wide range of different symptoms in infected host plants, including mosaic, stripe, mottling, vein clearing, vein banding, ringspots, necrotic or chlorotic lesions, leaf curling, flower breaking, necrosis, stunting and effect yield losses in crops. In monocotyledonous species, longitudinal chlorotic or necrotic leaf streaks are common while in dicotyledonous species common symptoms include chlorotic vein banding, mosaic, mottling, necrosis, and/or distortion of leaves. The severity of the symptoms depends on particular virus strain, the specific host genotype, vectors and environmental conditions (Matthews, 1991; Shukla *et al*, 1994).

More than 200 species of aphids spread Potyviruses and most are from the subfamily *Aphidinae* (genera *Macrosiphum* and *Myzus*). *Myzus persicae* (family *Aphididae*) known as the green peach aphid, acts as a vector for the transmission of Potyviruses members and other mosaic viruses. *M. persicae* has been implicated in the transmission of over 180 plant viruses. This aphid is globally distributed, located in all but prominently present in coldest terrestrial habitats. It has one of the broadest host ranges, having been recorded from species of nearly 130 plant families.

PCR primers based on conserved sequences can be for the identification and screening of Potyviruses members for example conserved motifs (WCIEN and QMKAA boxes) of the CP (coat protein) region of Potyvirus (Bateson and Date, 1995; Gibbs and Mackenzie, 1997).

The plant samples from the different region of Northern India often show Potyvirus symptoms i.e. Mosaic, curling, stunting of leaves, ringspot, chlorosis and necrosis in plants. PCR primer based on highly conserved sequences can be used to clone and sequence core region of CP gene and other Potyviral genes, this sequences are sufficient to classify virus isolate.

CP gene sequence analysis along with other Potyviral genes suggested that remarkable genetic variation among their geographical isolates.

According to sequence homolology analysis of CP region we can identify and classify distinct members and their isolates of Potyvirus genus, to develop evolutionary tree and report new diseases.

With the development of reliable computational recombination detection tools and the increasing number for available genome sequences, many studies have reported evidence of recombination in a wide range of virus genera. The computational analysis suggested that interspecific recombination has resulted in remarkable diversity among Potyviruses.

Aims of the study

This thesis focuses on Molecular detection and characterization of potyvirus isolates infecting commercially important Crop plants from Shekhawati region of Rajasthan.

The precise objectives of this research work are as follows:

1. Survey for the Potyvirus infecting economical crop plant species in Rajasthan, India.
2. Standardization of RNA isolation, c-DNA preparation and Polymerase chain Reaction (PCR) by potyvirus specific primers.
3. Cloning and sequencing of coat protein gene and other genes of potyvirus.
4. Sequence analysis of coat protein gene (CP) and construction of Phylogenetic tree.
5. Recombination analysis and genome relationships study.
6. To develop the degenerated primer for the identification of potyvirus

REIVEW OF LITERATURE:

Total number of Potyviruses increased to 111 definitive species and 86 tentative species (Fauquet *et al.*, 2005). In addition, new viruses (Ciuffo *et al.*, 2006; Turina et al., 2006), new strains (James and Varga, 2005; Tan et al., 2005) and new recombinants (Martín *et al.*, 2006; Matic *et al.*, 2006; Zhong *et al.*, 2005) are appearing continuously. The *Potyvirus* genome consists of a single plus-stranded, positive sense RNA molecule of ~10 kb, surrounded by about 2000 units of CP. The 5′ end of the genome carries a viral protein genome linked protein VPg and a polyadenine (poly-A) tail covalently bound to the 3′ end. The genome contains a single long ORF which is translated into a large 340-370 kilodalton Polyprotein and then cleaved by virus-encoded proteinases into ten functional proteins: P1 protein, helper component protein (HC-Pro), P3 protein, 6K1 protein, CI protein, 6K2 protein, nuclear inclusion a linked VPg protein (NIa-VPg), nuclear inclusion a protein (NIa-Pro), nuclear inclusion b protein (NIb) and CP (Riechmann *et al.*, 1992; Shukla et al., 1994).

P1 is a proteinase. P1 is the most variable region of the genome, with the exception of the C-terminal (Adams *et al.*, 2005; Ureuqui-Inchima *et al.*, 2001). P1 is the serine proteinase that self cleaves P1/Hc-Pro junction at a conserved YS or FS motif (Adams et al., 2005; Verchot et al., Yang *et al.*, 1998). P1 binds non-specifically to the RNA and it has been suggested that P1 may be involved in viral movement (Brantley and Gunt, 1993).

HC-Pro is a multifunctional protein required for viral acquisition by the vector, systemic and cell-to-cell movement and suppression of PTGC. Highly conserved K (I/L) (T/S) C motif (known as KITC motif), was required for interaction of HC-Pro with the aphids. A PTK motif in the core region of HC-Pro was identified as important for virion-binding. The C-terminal region of HC-Pro has a cystein-proteinase like activity required for auto cleavage between HC-Pro and P3 at its 3' terminal region.

CI is a major component of the replication complex. It is a important component of associated replication complex. In this case CI can unwind RNA duplexes with 3' overhangs in the 3' to 5' direction (Fernandez *et al.*, 1995; 1997). 6K1, in association with P3, carries a determinant for the pathogenicity. 6K2 is required for genome replication because it anchors the replication apparatus to the endoplasmic reticulam (Schaad *et al.* 1997a).

The VPg is the N-terminal part of NIa and involved in Potyvirus genome replication. VPg is reported to interact with host translational initiation factors like eIF4E and eIF (iso) 4E. The N-terminal region of NIa encodes the VPg and C-terminal region encodes for trypsin like protease (NIa-Pro). NIa-Pro cleaves the junctions of P3/6K1, 6K1/CI, CI/6K2, 6K2/VPg, VPg/NIa-Pro, NIa-Pro/Nib and Nib/CP. NIb is a RNA dependant RNA polymerase which has a poly (U) polymerase activity.

CP (coat protein) is a well known Potyviral protein. N-terminal of CP is responsible in aphid transmission of Potyviruses. CP gene's DAG motif interacts with Hc-Pro gene's KITC motif in aphid transmission. Core region of CP gene is involved in encapsidation. A common feature of plant viruses, differentiating them from human and animal viruses, is broad host range.

More than 700 plant viruses have been recognized, most of which have a broad host ranges (Strange, 1993). Some potyviruses do have a relatively wide host range, *Watermelon mosaic virus* (WMV), for example, infects plants in 23 families of dicotyledonous species, and occurs naturally in cucurbit and legume crops (Purcifull *et al.*, 1984). The hosts of *Potyvirus* listed by Brunt *et al.* (1996a) included 503 plant species belonging to 59 plant families, most of which are economically important plants. In addition, new hosts are continuously reported (Aılı-Sokmen *et al.*, 2005; Chen *et al.*, 2003; Csorba *et al.*, 2004; Morris *et al.*, 2006; Parry and Persley, 2005).

Cucumber mosaic virus has an exceptionally wide host range and can infect >200 species belonging to 20 plant families (Chamberlain, 1954), and the globally distributed *Barley yellow dwarf virus* can infect over 150 species of the Poaceae, including all the major cereals such as wheat, barley, oats, rye, rice and maize (Strange,1993). Recent and current studies continue to discover more and more new hosts such as *Apple stem grooving virus* in *Actinidia chinensis* (Clover *et al.*, 2003), *Turnip mosaic virus* in Calla lily (Chao et al., 2003), *Zucchini yellow mosaic virus* in cucurbitaceous sp. (Csorba *et al.*, 2004).